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Whole-Body Lifetime Occupational Lead Exposure And Risk Of Parkinson's Disease

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Key words: case-control, chronic toxicity, K-X-ray fluorescence, lead exposure, neurodegeneration, occupational exposure, Parkinson's disease.

Abbreviations:

Cadmium (Cd)

Confidence Interval (CI)

Corporate Data Store (CDS)

Gigabecquerel (GBq)

Industrial Hygienist (IH)

International Classification of Diseases, version 9 (ICD-9)

Kiloelectron Volt (keV)

K-series X-Ray Flourescence (K-XRF)

Lead (Pb)

Microgram per deciliter (μg/dL)

Millicurie (mCi)

Mini-Mental State Exam (MMSE)

Odds Ratio (OR)

Parkinson's Disease (PD)

Permissible Exposure Level (PEL)

Physiologically-based Pharmacokinetic (PBPK)

Outline:

Abstract

Background: Several epidemiological studies have suggested an association between Parkinson's disease and exposure to heavy metals using subjective exposure measurements. **Objectives:** We investigated the association between objective chronic occupational lead (Pb) exposure and the risk of Parkinson's disease (PD). **Methods:** We enrolled 121 PD patients and 414 age, gender, and race, frequency-matched controls in a case-control study. As an indicator of chronic lead exposure, we measured concentrations of tibial and calcaneal bone lead stores using ^{109}Cadmium (Cd) excited K-series X-Ray Fluorescence (K-XRF). As an indicator of recent exposure, we measured blood lead concentration. We collected occupational data on participants from age 18 until age at enrollment and an industrial hygienist determined the duration and intensity of environmental lead exposure. We employed physiologically-based pharmacokinetic (PBPK) modeling to combine these data and estimated whole-body lifetime lead exposures for each individual. Logistic regression analysis produced estimates of Parkinson's disease risk by quartile of lifetime lead exposure. **Results:** Risk of PD was elevated by more than two fold, OR=2.27 (1.13-4.55; P=0.021) for individuals in the highest quartile for lifetime lead exposure relative to the lowest quartile, adjusting for age, gender, race, smoking history, coffee, and alcohol consumption. The associated risk of PD for the second and third quartiles were elevated but not statistically significant at the α=0.05 level. **Conclusions:** These results provide an objective measure of chronic lead exposure and confirm our earlier findings that occupational exposure to lead is a risk factor for Parkinson's disease.

Introduction

Parkinson's disease (PD) is a neurologic movement disorder in which neurons of the substantia nigra, which are responsible for dopamine production, attenuate in number or become functionally impaired. Resultant symptoms include resting tremor in the extremities and head, limb and/or trunk rigidity, bradykinesia, and postural instability. Although the primary cause of the destruction or failure of substantia nigra cells is unknown, mounting evidence exists for both environmental and genetic determinants. A growing body of evidence suggests that heavy metal cations stimulate free radical formation in the brain and can lead to neurodegeneration via peroxidative damage to the cell membrane (Sandhir et al. 1994). Increasing levels of heavy metal cations stimulate the conformational changes which can lead to fibrillation of recombinant α-synuclein. The aggregation and fillibration of α-synuclein provoked by the presence of heavy metal cations could directly cause the intracellular protein inclusions that are observed in the substantia nigra of PD patients (Uversky et al. 2001). Results from several epidemiological studies further support an association between PD and exposure to heavy metals (Aquilonius and Hartvig 1986;Rybicki et al. 1993;Tanner et al. 1989). Our previous results demonstrated a two-fold increase in the risk of Parkinson's disease among workers with more than 20 years of occupational exposure to lead. Associations of such chronic occupational exposure to combinations of lead-iron and lead-copper were even more robust (Gorell et al. 1997).

While several studies have examined the relationship between blood lead levels and environmental lead exposure, results from blood lead analysis are not noteworthy. Research into long-term or distant past lead exposures is confounded by body's ability to purge metals quickly from the blood stream. Because the half-life of lead in blood is approximately one month, blood lead levels only reveal if the individual has experienced a relatively current exposure to

environmental lead. Thus, blood lead levels are indicative of only acute and recent exposures to lead rather than representing a chronic and/or extended exposure history. Rather than within circulating blood, the main repository for chronic lead stores in the body is within bone (Hu et al. 1998). Lead is stored in the bone by replacing calcium of the hydroxy-apatite crystals of the bone mineral. Through a continual process of bone remodeling, synthesis and reabsorption, lead is released into the blood stream and circulates throughout the body. In the brain, lead diffuses easily across the blood brain barrier and binds to sulphydryl groups, resulting in lead neurotoxicity that leads the neurodegeneration via intracellular oxidative damage (Bradbury and Deane 1993;Popenoe and Schmaeler 1979;Quinlan et al. 1988;Rossman 2006;Sandhir et al. 1994).

Additionally, while the half life of lead in blood is very short, the half-life of lead in bone is measured in years and decades, depending on the type of bone—the hard bone of the tibia (half life of approximately 20 years) releases lead more slowly over time than the soft, spongy bone of the calcaneus (half-life of up to 10 years)—and the individual's metabolic rate of leaching and clearance (Patterson C.C 1980). Measuring lead concentration in bone using K-shell X-Ray Fluorescence (K-XRF) provides a proxy for current whole-body lead content, independent of whether the exposure is current (Hu et al. 1991;Todd and Chettle 1994). While K-XRF measurements estimate the current level of blood stores in the body, they can be used to construct an indirect assessment of an individual's past exposures to lead. If the exposure to lead was experienced at some time in the past, then the individual's body will have cleared some amount of lead between the last period of exposure and the time of the K-XRF measurement. This clearance rate of lead from bone is predictable and can be used in a physiologically-based pharmacokinetic (PBPK) model to estimate the level of lead in the body at the actual time of the

exposure. Kinetic modeling allows for a more complete picture of the individual's lifetime occupational lead exposure by combining current body stores, as detected by bone lead levels; current exposures, as indicated by blood lead levels; and both current and past environmental exposures, as reported by the individual and qualified by industrial hygienist assessment to assess the timing and intensity of the exposure (Leggett 1993).

Herein, we further evaluated the risk of PD in response to the total body lead burden. To accomplish this, we conducted a case-control study with the following objectives: 1) Integrate historical exposure information with current measurements of cortical and trabecular bone lead obtained by current K-X-ray fluorescence (K-XRF) and blood lead; 2) Reconstruct the lead exposure history using physiologically-based pharmacokinetic models, and 3) Use the resultant estimate of the net body lead burden over time (dose) to calculate a quantitative relationship between the life-time exposure and the risk of Parkinson's disease.

Materials and Methods

Setting and Study Population. Study participants were patients who had received their primary health care services between 1995 and 1999 from the Henry Ford Health System (HFHS), the second largest health care provider in southeastern Michigan. We identified potential study participants age 50 years and older from a database containing data for a total 127,742 patients using the internal Corporate Data Store (CDS) computerized billing system within HFHS (Figure 1). We implemented a multistep screening process for the ascertainment of PD diagnosis. First, we used the ICD-9 codes of 332 and 332.0 to identify potential cases from the CDS and identified a total of 2,678 potential cases. In the second step of screening, we reviewed electronic and/or hard copies of medical records of the 2,678 individuals for a diagnosis of idiopathic PD within the previous 5 years. Of these, a total of 249 individuals had

confirmed clinical diagnoses of PD. We randomly identified potential controls from the CDS and frequency-matched them on gender, race, and age of case at the time diagnosis of PD (±5 years). Frequency-matching reduces the primary effects of these variables in the analysis phase. We reviewed the medical records of potential cases to exclude patients with secondary Parkinsonism, stroke-induced Parkinsonism, and Parkinsonism as a result of head injury, brain tumor, encephalitis, neuroleptic use, carbon monoxide intoxication, Huntington's disease, Wilson's disease, essential or intention tremor, dementia, verbal aphasia, seizure disorder, psychosis or depression, substance abuse, and those patients with hydrocephalus. One of the investigators (J.G.) and two registered nurses in the Department of Neurology reviewed all of the medical records and made the final screening eligibility judgment.

We mailed a recruiment packet containing a letter explaining the purpose of the study, a one-page sheet of Frequently Asked Questions about PD and the research study, and a brochure describing the procedure of bone lead measurement using K-series X-Ray Fluorescence (K-XRF) to a total of 249 cases and 1,519 potential control volunteers. A week after mailing, trained interviewers made follow-up telephone calls and invited the potential participants to participate in the study. Those individuals who agreed to do so, underwent a telephone administered shortened version of the Mini Mental State Exam (MMSE) as the final step in the screening process. A total of 228 eligible cases and 1,334 eligible controls were asked to participate in the study and a total of 121 cases (53.1%) and 414 controls (31.0%) agreed and were enrolled in the study. Participants were scheduled for a clinic visit during which they were administered the full version of the MMSE, the K-XRF procedure, had blood drawn for laboratory analysis of lead, were interviewed for potential occupational exposure to lead and for other possible risk factors for PD. To rule out patients with dementia, all final participants had a

full MMSE score greater than or equal to 24 (American Psychiatric Association 1994;Anthony et al. 1982;Folstein et al. 1975). Participants gave written informed consent, were minimally compensated, and the study was approved by the Institutional Review Board within the Henry Ford Health System.

Lead Exposure Assessment. During a face-to-face interview using a modified version of the lead exposure questionnaire instrument of Kosnett et al. (1994), study participants described the types and duration of occupations held throughout their adult life (age 18 to present) (Kosnett et al. 1994). One of the investigators (G.K.), an industrial hygienist (IH), blinded to the status of the study participant, ranked the probability of lead exposure and the intensity of exposure as high, medium or low for each occupation lasting greater than six months. High probability exposures occurred when the activity performance exceeded 90% of the work period and involved direct handling of lead containing materials, or the liberation of lead-containing fumes, dusts, or liquids in the vicinity of the subject. Moderate probabilities of lead exposure (10-90% of the time) and low probabilities (<10% of the time) were associated with "occasional" (moderate) or "rare or never" (low) contact with leaded materials or lead-containing fumes, dusts or liquids. The intensity of exposure was rated high if the probability of exposure was also high and the permissible exposure level (PEL, National Institute for Occupational Safety and Health) standard was exceeded >50% of the time, giving a high score using the paradigm of Sharp et al (Sharp et al. 1991). Moderate intensities of exposure were associated with 10 to 100 % of the PEL and a moderate level by Sharp et al, and low intensities were at < 10% of the PEL with a low exposure score according to Sharp et al. We then combined probability and intensity scores with duration for each occupation so that each subject had a cumulative lead exposure history over time from age 18 to the time of recruitment.

Bone Lead Measurement by K-X-Ray Fluorescence (K-XRF). We performed K-XRF measurement of bone lead on each subject during 40 minutes of exposure to the left tibia and calcaneus to a sealed 1.1GBq (30 mCi) ^{109}Cd source, placed in a commercial detector (Canberra Industries, Meriden, CT), 2 cm from each measured bone. The reflected 88 keV γ-rays were amplified and digitized, and we collected the energy spectrum on a multi-channel analyzer. The amplitudes of α1 and β1 K-series X-rays and their ratios to the amplitude of the coherent scatter peak were determined for each human bone spectrum and evaluated calibration lines generated from a set of 10 standard lead-doped plaster-of-paris phantoms with lead concentrations ranging from 0 to 200μg/g. Using a non-linear Chi-squared minimization procedure, we fit models of the spectral features to each individual's data (Bevington P.R. 1969;Marquardt 1963). The amplitudes of the lead X-rays and of the coherently scattered 88 keV γ-rays were extracted for further analysis. We based quantity assessment on independent estimates of the amplitudes of α_1 and β_1 K-band x-rays and their ratios to the amplitude of the coherent scatter peak, giving two calibration lines, corresponding to α and β x-rays. The same 2 ratios were determined for each human bone spectrum and evaluated against the appropriate calibration lines, making due allowance for the difference in coherent scattering between bone and plaster-of-Paris. We calculated the result for an individual subject as the inverse variance of the weighted mean of the 2 ratios. At the time of K-XRF measurement, we drew 5 ml of venous blood from each subject for determination of blood lead levels. Samples were analyzed by atomic absorption spectroscopy at Henry Ford Hospital in Detroit, MI (Miller et al. 1987).

Physiologically-Based Pharmacokinetic Modeling of Lifetime Lead Exposure. We integrated lead exposure duration and intensity data with current measures of blood and skeletal lead concentrations using the International Commission for Radiation Protection (ICRP)

physiologically-based pharmacokinetic (PBPK) model (Leggett 1993). We developed an algorithm to convert exposure questionnaire information objectively for use in biokinetic modeling. Each occupational activity that involved risk of lead exposure was assigned the probability and intensity scale values of the activity (low, medium, and high), converted to 1, 2, or 3, respectively. The daily lead input to the model from a particular activity was then determined using the following formula:

[1]

$$\textit{Daily Lead Input} = P \times \textit{Intensity} \times \frac{\textit{(Activity hours)}}{40} \times 10\ \mu g\ \textit{lead/day}$$

Where P = Probability of lead exposure because of job classification; Intensity = Intensity of exposure; Activity hours = number of hours devoted to the activity per 40 hours of work week. The value of 10μg lead/day was derived from the assumption that an individual working 40 hours per week in a "high probability/high intensity" exposure activity should have a blood lead level just above 40 μg/dL (Patterson C.C 1980). A calculation of 3 x 3 x (40/40) x 10 = 90 μg/dL gave sufficient daily lead input above the baseline to result in blood lead levels of 42 μg/dL during this activity. We then calculated the total life time exposure for each individual by adding the lead exposure questionnaire-derived values for each occupation plus the baseline lead input at that subject's age at enrollment. Next, we incorporated age-dependent changes in bone remodeling rates and skeletal mass above the age of 60 and scaled the biokinetic model to an individual subject's body weight, rather than to the commonly used 70 kg reference man (ICRP 1996).

We adjusted the intensity of lead exposure (μg lead/day) in the model for the current blood lead level, and trabecular (calcaneus) and cortical (tibia) bone lead concentration to minimize the model fit error using the formula:

[2]

$$\textit{Model fit error} = \frac{[(\text{K-XRF}_{tibia} - \text{model}_{cortical\ bone}) \times 2 + (\text{K-XRF}_{calcaneus} - \text{model}_{trabecular\ bone})]}{3}$$

This calculation weights the model fitting to the K-XRF measurement of the tibia (cortical bone) because of the longer half-life of lead in cortical bone, and because the error of K-XRF measurement is smaller than for trabecular bone. The use of this equation provides an objective description of the model's fit to limited data. Input K-XRF estimates of bone lead ranged from 4.5μg/g (μg lead/g bone) to 54.4 μg/g with a mean of 12.5 +/- 7.8μg/g for tibial and from 9.3μg/g to 64.4μg/g with a mean of 20.5 +/- 10.2μg/g for calcaneal measurements. The resulting model fit error ranged from -37.5 to 18.5μg/g and produced a mean error of -4.0 +/- 6.7μg/g.

Statistical Methods. To assess the distribution of exposures among case and control participants, we used chi-square analysis. To assess the relationship of lead exposure to the presence of PD we used multiple logistic regression techniques. The dependent variable was the presence or absence of PD. Odds ratios and their associated 95% confidence intervals were calculated. For all models a p-value <0.05 was considered statistically significant. For lead exposure, we calculated the whole-body lifetime lead exposure as described above and categorized the participants into four quartiles based on the mean exposure per year for the control group: first quartile (0 to 40.04 microgram lead storage per gram of bone, μg/g), second (40.05 to 57.43 μg/g), third (57.44 to 80.80 μg/g), and fourth (greater than or equal to 80.81 μg/g). Logistic regression analysis was run with three indicator variables and the first quartile of exposure serving as the reference group. The range of whole-body lifetime lead exposure measurements was skewed by several large exposure values, making the data difficult to model as a continuous variable. A log-transform of the data modeled well and these results are also included below.

Results of the K-XRF measurements were also examined via logistic regression using either tibia or calcaneus lead exposure as the independent variable to be associated with PD. Tibia bone lead exposure was categorized into quartiles based on the exposure of the control group as follows: first (0 to 5.91 μg/g), second (5.92 to 10.40 μg/g), third (10.41 to 15.50 μg/g) and fourth (≥ 15.51 μg/g). Finally, calcaneus bone lead exposure was categorized into quartiles based on the exposure of the control group as follows: first (0 to 11.70 μg/g), second (11.71 to 19.07 μg/g), third (19.08 to 25.28 μg/g) and fourth (≥ 25.29 μg/g).

In adjusted logistic regression models, we included the matching covariates of age, gender, and race. While we modeled age as a continuous variable, gender and race were both included as dichotomous variables of male/female and white/non-white, respectively. Since smoking history, coffee consumption, and alcohol consumption have been reported on extensively as effect modifiers in determining PD risk, we also included these factors in adjusted models. We calculated pack-years of smoking history (i.e., packs of cigarettes per day multiplied by years smoked), and categorized smokers into three groups: none (0 pack-years), mild to moderate (>0 to 30 pack-years), and heavy (>30 pack-years). The analysis thus ran two indicator variables with non-smokers serving as the reference group. We defined coffee consumption history as the number of coffee-years where a coffee-year was defined as one cup of coffee per day for one year. We categorized coffee drinkers, using the median coffee consumption for the control group, as low (>0 to 112 coffee-years) and high (greater than 112 coffee years). Two indicator variables were used in the logistic regression analysis and no coffee consumption served as the reference group. For alcoholic consumption, we defined a "drink-year" as the intake of one "drink" of alcohol per day for one year. We defined a "drink" of alcohol as follows: liquor (30 ml), one can of beer (360 ml), or one glass of wine (120ml). We

classified participants as nondrinkers (0 drink-years), mild to moderate drinkers (>0 to 10 drink-years), or heavy drinkers (>10 drink-years). Two indicator variables were used in the logistic regression analysis and non-drinkers served as the reference group.

Results

We enrolled 535 non-demented men and women aged 50 and over. The average age of participants in the study was 69.9 ± 8.2 years and did not differ from non-participants (69.7 ± 9.1), P=0.69 (Student's T-test). Also, race did not differ significantly between participants (85.8% white) and non-participants (83.2% white), P=0.19 (Mantel-Haenszel Chi-Square). Gender, however, did differ significantly between participants (56.6% male) and non-participants (45.7% male), P=0.001 (Mantel-Haenszel Chi-Square). This difference resulted from the combination of both a higher incidence of PD within males and our inability to recruit healthy males as effectively as healthy females for participation.

Of the 535 participants, 121 had a verified idiopathic diagnosis of Parkinson's disease and 414 were healthy age, gender, and race, frequency-matched controls (Table 1). This included 303 (56.6%) males and 232 (43.4%) females, 460 (86.0%) whites and 75 (14.0%) non-whites. Even with a recruitment protocol designed for frequency matching, a statistical difference was observed for age and race. Individuals aged 70 and older (P=0.001) and whites (P=0.038) were under-represented among healthy controls when compared to PD cases. Cases had an average age of 72.3 (median 73.1) years and controls had an average age of 69.7 (median 70.0) years. While males were also somewhat underrepresented among healthy controls, the difference from cases did not reach statistical significance. To account for the difficulty in balancing recruitment among these groups, the final logistic regression models included adjustment for these variables. Distributions of the covariates for smoking history, coffee

consumption, and alcohol consumption can be seen in Table 1. Patients with Parkinson's disease were more likely to be non-smokers or to be lighter smokers than healthy controls (P=0.002). Controls were nearly twice as likely to be heavy smokers (>30 pack-year history). PD patients were also less likely to be coffee drinkers than controls (P=0.007). No difference in alcoholic consumption was observed between patients with Parkinson's disease and healthy control volunteers.

Participants were categorized into quartiles of exposures individually for categories of whole-body lifetime lead exposure, K-XRF calcaneal bone lead measurement, and K-XRF tibial bone lead measurement, as described in Materials and Methods. Among patients with Parkinson's disease whole-body lead concentrations were lowest in the first quartile (14.9%) and highest in the fourth quartile (32.2%), yielding a positive indication for an association between lead exposure and PD (P-trend = 0.036). Delegation of tibial K-XRF measurements into quartiles also reveals a significant trend from the first to fourth quartile (P-trend=0.012). A statistically significant trend is not seem, however, when applied to quartiles of calcaneal K-XRF measurements (P-trend=0.275).

Results from logistic regression modeling of quartiles of whole-body lifetime lead exposure showed a statistically significant elevation of risk of Parkinson's disease for those in the fourth (highest exposure) quartile compared to those in the first (lowest exposure) quartile (Table 2). The estimated odds ratios (95% confidence interval; P-value) indicates an elevated risk of PD for those in the fourth quartile, adjusting for age, sex, race, smoking, and coffee and alcohol consumption, OR = 2.27 (1.13, 4.55; P = 0.021). Individuals who experienced the highest quartile of exposure were twice as likely to have Parkinson's disease than those in the lowest quartile of exposure. The risk estimates for tibial and calcaneal K-XRF measurements of

bone lead concentrations alone (without incorporation of duration and intensity of occupational exposure data or blood lead levels) were elevated in increasing quartiles of exposure, but these data did not reach statistical significance. In the adjusted lifetime lead exposure model, moderate, OR = 0.58 (0.35, 0.95, P = 0.029), and heavy, OR = 0.33 (0.17, 0.65, P = 0.001) levels of smoking were associated with reduced risks of PD. Also, mild to moderate, OR = 0.41 (0.22, 0.75, P = 0.004) and heavy, OR = 0.36 (0.19, 0.68, P = 0.002) coffee drinkers displayed a reduced risk of PD as well. Neither mild to moderate nor heavy alcohol consumption was significantly associated with Parkinson's disease risk.

We then analyzed the log transformation of the continuous lifetime lead exposure data by logistic regression. Adjusted, whole-body lifetime lead remained significant, OR = 1.74 (1.10, 2.75, P = 0.018), while K-XRF measurements of tibia, OR = 1.47 (0.99, 2.20, P = 0.059) and calcaneal, OR = 1.53 (0.93, 2.54, P=0.097) did not reach statistical significance. Stratified analysis pointed to a stronger association between lifetime lead exposure and PD risk for those older than, OR = 2.17 (1.16, 4.06, P = 0.015), vs. those younger than, OR = 1.30 (0.62, 2.70, P = 0.49), the median age (70.7 years), but testing for an interaction did not show a statistically significant difference between the odds ratios, P = 0.24. Similarly, stratified analysis by gender suggested that the association of lifetime lead exposure to PD risk was higher in females, OR = 2.23 (1.05, 4.76, P = 0.038) than in males, OR = 1.53 (0.83, 2.80, P = 0.17), but an interaction test showed that this difference was not statistically significant, P = 0.50.

Independent of PD diagnosis, age, sex, smoking, coffee, and alcohol were associated with whole-body lifetime lead exposure (data not shown) while race was not. Even so, the estimate of PD risk changed very little before and after adjustment for these covariates. The logistic model for whole-body lifetime lead exposure had 99% and 98% power to detect a difference in the

unadjusted and adjusted models, respectively. The logistic model for tibial K-XRF measurement of lead exposure had 92% and 72% power to detect a difference in the unadjusted and adjusted models, respectively. The logistic model for tibial K-XRF measurement of lead exposure had 57% and 79% power to detect a difference in the unadjusted and adjusted models, respectively.

Discussion

Occupational exposure to heavy metals is associated with the risk of PD (Gorell et al. 1997). Assessment of blood lead concentration as a proxy for chronic or distant past exposure is inconclusive because of body's ability to purge metals quickly from the blood stream. We employed the K-X-Ray Fluorescence technology to improve the assessment of chronic lead exposure by measuring long-term lead stores in the body. The use of K-X-Ray Fluorescence represents an advancement in methodology beyond our earlier work which subjectively assessed occupational exposure to lead by an experienced industrial hygienist (Todd and Chettle 1994;Uversky et al. 2001). Additionally, we employed physiologically-based pharmacokinetic (PBPK) modeling which combined the bone lead concentration with occupational history and blood lead concentration to estimate whole-body lifetime lead burden. Our findings show that higher levels of lifetime exposure to lead is associated with risk of Parkinson's disease and confirms previous findings that that prolonged occupational exposure to lead increases the risk of PD by more than two fold (Gorell et al. 1997). This study agrees with the findings of several other epidemiological studies and further supports an association between PD and exposure to heavy metals (Aquilonius and Hartvig 1986;Rybicki et al. 1993;Tanner et al. 1989).

Recall bias is a primary source for error in the study of occupational exposure to heavy metals. This can manifest as either omission of exposure or exaggeration of duration and/or intensity of exposure. In an effort to reduce recall bias we supplemented the participant's self-

report with the experience of the industrial hygienist (IH) to determine if lead was used in a specific occupation. Also, by focusing primarily on occupational exposures, verifiable by industrial hygienist, rather than environmental and recreational exposure, we further reduced the likelihood of spurious findings. Another limitation presented herein involves the failure to accurately match cases and controls on age and gender. Individuals aged 70 and older were underrepresented in the control group compared to cases. Adjustment for age in the logistic model testing for the association of lead exposure on Parkinson's risk did appreciably alter the effect size or the significance level. The results of stratified analysis point to age as being an effect modifier for the association of lead exposure to PD risk, but not the source of the effect itself. Similarly, males were underrepresented in the control group compared to cases. Here also, the adjustment for gender did not significantly change the effect size or significance level of the association between lead exposure and risk of PD. Stratified analysis showed elevated odds ratios in both males and females that, while not significantly different, were stronger in females. Participants were more male than non-participants and may have resulted in a further source of error. Since the association between lead exposure and Parkinson's disease was stronger in females, it might be expected that the overall association in the general population be stronger than that reported above.

This work supports the hypothesis that lead plays a role in the etiology of Parkinson's disease in exposed individuals. While the biochemical mechanism of lead neurotoxicity is not completely understood, a growing body of evidence suggests that metal cations of lead, iron, and aluminum stimulate free radical formation which results in neurodegeneration via peroxidative damage to the cell wall. Sandhir et al. (1994), observed that increasing lead concentration in rat brain produces heightened levels of lipid peroxidation and decreased activity of neuroprotective

antioxidant enzymes and acetylcholinesterase. The authors suggested that lipid peroxidation eventually can lead to neuronal cells death through deterioration of the cell-membrane. Uversky, et al. (2001), using in vitro models of human brain cells, found that increasing levels of heavy metal cations stimulate the conformational changes which can lead to fibrillation of recombinant α-synuclein. The authors argue that the aggregation and fibrillation of α-synuclein provoked by the presence of heavy metal cations could directly cause the intracellular protein inclusions that are observed in the substantia nigra of PD patients. Quinlan et al. (1988), observed that while lead ions alone did not induce peroxidation, they did accelerate the rate of peroxidation caused by iron ions. The current study provides additional objective evidence to support the hypothesis that long-term exposure to heavy metals, such as lead, contributes to the accumulation of peroxidative damage and neurodegenerative cell death that is observed in Parkinson's disease.

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Table 1. Distribution, N (%), of matched variables and potential confounders in the study.

Matching Variable	Level	Cases N=121		Controls N=414		P-value [a]
Age	50-59	9	(7.4 %)	60	(14.5%)	0.001 [b]
	60-69	27	(22.3 %)	147	(35.5%)	
	70-79	63	(52.1 %)	166	(40.1%)	
	80+	22	(18.2 %)	41	(9.9%)	
Gender	Male	76	(62.8 %)	227	(54.8%)	0.119
	Female	45	(37.2 %)	187	(45.2%)	
Race	White	111	(91.7 %)	349	(84.3%)	0.038 [c]
	Non-White	10	(8.3 %)	65	(15.7%)	
Smoking	None	70	(57.9 %)	166	(40.1 %)	0.002 [b]
	Mild to Moderate	36	(29.8 %)	158	(38.2 %)	
	Heavy	15	(12.4 %)	90	(21.7 %)	
Coffee	None	30	(24.8 %)	53	(13.0 %)	0.007 [b]
	Mild to Moderate	46	(38.0 %)	180	(44.1 %)	
	Heavy	45	(37.2 %)	175	(42.9 %)	
Alcohol	None	20	(16.5 %)	53	(13.0 %)	0.544
	Mild to Moderate	45	(37.2 %)	168	(41.1 %)	
	Heavy	56	(46.3 %)	188	(46.0 %)	

[a] Chi-square.
[b] p-value < 0.01.
[c] p-value < 0.05.

Table 2. Risk of PD by whole-body lifetime lead exposure and K-XRF point measurements od lead exposure.

Variable	Unadjusted OR	(95% CI)	P-value	Adjusted [a] OR	(95% CI)	P-value
Whole Body						
second quartile	1.87	(0.99, 3.52)	0.052	1.90	(0.97, 3.71)	0.060
third quartile	1.67	(0.87, 3.18)	0.121	1.71	(0.86, 3.41)	0.125
fourth quartile	2.15	(1.15, 4.00)	0.016 [b]	2.27	(1.13, 4.55)	0.021 [b]
Tibia						
second quartile	0.90	(0.47, 1.73)	0.762	0.87	(0.43, 1.75)	0.691
third quartile	1.52	(0.84, 2.75)	0.165	1.33	(0.70, 2.52)	0.387
fourth quartile	1.81	(1.02, 3.22)	0.044 [b]	1.62	(0.83, 3.17)	0.160
Calcaneus						
second quartile	1.67	(0.92, 3.02)	0.092	1.71	(0.91, 3.20)	0.094
third quartile	1.18	(0.63, 2.22)	0.604	1.12	(0.57, 2.22)	0.737
fourth quartile	1.62	(0.89, 2.94)	0.113	1.50	(0.75, 3.00)	0.253

[a] adjusted for age, sex, race, smoking, coffee and alcoholic consumption.
[b] p-value < 0.05.

Figure Legend

Figure 1. Diagram of case and control selection, recruitment, and enrollment.

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